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1. Your reference	AHB/CP5854112	
2. Patent application number *(The Patent Office will fill in this part)*	04 JUL 2000	**0016441.8**
3. Full name, address and postcode of the or of each applicant *(underline all surnames)*	PHARMAGENE LABORATORIES LTD 2 ORCHARD ROAD ROYSTON HERTFORDSHIRE SG8 5HD UNITED KINGDOM	
Patents ADP number *(if you know it)*		7255530001
If the applicant is a corporate body, give the country/state of its incorporation	GB	
4. Title of the invention	THERAPEUTIC METHOD	
5. Name of your agent *(if you have one)*	MEWBURN ELLIS	
"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	YORK HOUSE 23 KINGSWAY LONDON WC2B 6HP	
Patents ADP number *(if you know it)*	109006	

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8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*
a) any applicant named in part 3 is not an inventor, or
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YES

THERAPEUTIC METHOD

Field of the Invention.

The present invention relates to the treatment of cystic fibrosis with the hormone secretin or other secretin receptor ligands.

Background to the Invention.

Cystic fibrosis (CF) is the most common, fatal, autosomal recessive inherited disease, with over 7000 people currently diagnosed in the UK alone and approximately 30,000 in the United States. The incidence of CF is strongly dependent on ethnic background. Caucasian individuals with Northern European ancestry are most at risk exhibiting a probability of approximately 1 in 2500, based on a heterozygous carrier rate of about 1 in 25.

CF arises as a result of genetic mutation(s) in the gene of the cystic fibrosis transmembrane regulator (CFTR) chloride channel throughout the body. Such mutations in the CFTR lead either to incorrect folding of the protein and / or the lack of migration of the transcribed protein from the Endoplasmic Reticulum to the epithelial plasma membrane and subsequent loss of chloride (Cl-) channel function. This causes a cellular and luminal imbalance in fluid and electrolyte transport and volume within the lower respiratory tract of the CF lung, which reduces the constitution

of the mucus which in turn impairs mucociliary clearance and initiates the inevitable and persistent bacterial infections within the lung of CF patients. Different mutations give rise to CF symptoms of varying severity and correspondingly lead to variations in patient survival rates.

Over the last few decades, improved drug and physiotherapy treatments have improved patient survival time significantly, though average life expectancy is still short, currently around 30 years. There is therefore a continuing need to develop better treatment for this condition.

Secretin is a peptide hormone which is secreted from S cells in the proximal small intestine (especially the duodenum and jejunum) in response to acidic contents leaving the stomach. The structure of porcine secretin has been known for some time and it has been isolated from porcine intestine and has been found to be constituted by a peptide composed of 27 amino acid residues (Mutt, V., Jorpes, J. E. and Magnusson, S. (1970) Eur. J. Biochem., 15, 513-519). Moreover, it has been found that bovine and porcine secretins are identical, and are also similar to canine secretin.

Although bovine and porcine secretins behave identically with human secretin in some respects they are not structurally identical. These animal secretins differ from the human secretin at positions 15 and 16. An alignment of human, porcine and canine secretin is shown in Figure 1.

Secretin's physiological role is to stimulate water H_2O and bicarbonate HCO_3^- secretion from the pancreas, leading to the neutralisation of acidic chyme. Its actions are mediated via a seven transmembrane domain, G protein coupled receptor GPCR, a member of the glucagon-secretin-vasoactive intestinal peptide structurally related superfamily of GPCRs IUPHAR Receptor Compendium, 1998, for which the peptide exhibits nanomolar affinity. Secretin receptor stimulation mediates increases in intracellular cAMP, and the activation of protein kinase A PKA.

Secretin is currently approved by the FDA to diagnose gastrinoma and assess pancreatic function. Anecdotal reports from "off-label" use of secretin in pediatric autism suggest that it may improve both physiological and behavioural symptoms associated with autism, a disorder characterized by severely impaired communication, social skills and development see for example WO98/52593, US-A-6,020,310 or US-A-6,020,314. In March 2000 Repligen Corporation USA announced it had initiated a Phase II clinical trial with secretin in children with autism, with the Phase II trial sites including the Mayo Clinic, the University of Rochester Medical Center and the Southwest Autism Research Center in collaboration with Phoenix Children's Hospital.

Secretin has also been proposed for the prophylaxis of the aspiration pneumonia syndrome e.g. in EP0150760; AU3806485.

There are a wide number of reported synthetic and/or naturally

occurring secretin peptide analogues and fragments (referred to herein as "secretin receptor ligands") which exhibit a wide range of potencies, efficacies and selectivity for the secretin receptor. These include, but are not limited to mono / poly substituted secretin analogues, secretin fragments, substituted secretin fragments, reduced peptide bond analogues (Gardner et al, 1976; Gardner et al, 1979; Waelbroeck et al, 1981; Konig et al, 1984; Staun-Olsen et al, 1986; Robbertecht et al, 1988; Haffer et al, 1991). Also known are secretin-related receptor peptides, and associated analogues and fragments which exhibit affinity for the secretin receptor.

Disclosure of the Invention.

We have studied the expression levels of secretin receptor in tissue from patients with CF. We have surprisingly found that levels of secretin receptor mRNA in tertiary bronchus of CF patients are significantly elevated. This elevation is specific to CF, and not shared by patients with other lung disorders. The elevation was specific to tissue of the tertiary bronchus.

While not wishing to be bound by any one particular theory, we believe the action of secretin on ion movements in cells (see below) will counteract the effect of the CTFR deficiency associated with CF. Further, although the operation of the present invention does not rely upon any one particular theory, an explanation of the elevated levels of secretin receptor mRNA in tertiary bronchial tissue is that this is a response to the

ion imbalance experienced in these cells.

Accordingly, the present invention provides a method of treatment of cystic fibrosis in a patient suffering from CF, the method comprising administering to said patient an effective amount of a secretin receptor ligand.

The invention also provides the use of a secretin receptor ligand for the manufacture of a medicament for the treatment of cystic fibrosis.

Preferably, the secretin receptor ligand is secretin, particularly human secretin.

Brief Description of the Drawings.

Figure 1 shows an alignment of human, porcine and canine secretin.

Figure 2 shows differential expression of mRNA of the secretin receptor in control and CF lung regions.

Figure 3 shows mRNA expression of GAPDH in control and lung CF regions.

Detailed Description of the Invention.

Secretin Receptor Ligand.

As indicated above, the preferred secretin receptor ligand is human secretin (hSN). However, other mammalian secretins, such as bovine, porcine (pSN) or canine secretins may be used, as well as synthetic secretin receptor ligands such as those identified above.

Other secretin receptor ligands are well known in the art. Many such ligands are based on the sequence of a natural secretin (e.g. human or porcine secretin) but contain from 1 to 7 (more usually from 1 to 5, and often 1, 2 or 3) amino acid substitutions or deletions, particularly but not exclusively in the N-terminal region.

For example, Gespach et al (Peptides, 1986;7 Suppl 1:155-63) describe four synthetic secretin analogues including one corresponding to porcine secretin substituted at the N-terminus by sequence portions of vasoactive intestinal peptide (VIP), i.e. Ala4-Val5-pSN, together with Tyr1-Ala2-Glu3-pSN, Gln3-pSN, Phe1-Phe2-Trp3-Lys4-pSN. Konig et al (Gastroenterology, 1977, 72;797-800) describe Ala4-pSN. Gardener et al (Gasteroenterology 1976, 71;965-970) describe the secretin fragment SN5-27 and three variants thereof, (9Gln-SN5-27, 15Asn-SN5-27 and 9Gln-15Asn-SN5-27). 15-Lys-SN has also been described in the art (Gardener et al, Biochem. Biophys. Acta, 1979, 3;583). Haffar et al describe eight secretin variants with reduced peptide bonds (the -CONH- bond being replaced by -CH2-HN-) between one of the eight N-terminal peptide bonds. Robberecht et al (Pancreas, 1988, 3;529-535 describe secretin

fragments 2-27, 3-27, 5-27 and 7-27 and observed activity for secreting receptors. Konig et al Peptides, 1986, 7 Suppl 1:61-67 exchanged the N-terminal 5 amino acids of a secretin for the N-terminal pentapeptide sequence of human somatotropin releasing factor to provide 1-Tyr-2,4-diAla-5-Ile-SN, which showed secretin activity. Other active variants made were 3-L-Cystic acid-SN, 6-D-Phe-SN, 5-Allo-Thr-SN, and 1-Cys-6-Cys-SN.

The secretin receptor ligands described in the above literature, which is incorporated herein by reference, may all be used in the present invention, though those of skill in the art will appreciate that the above-cited references are not exhaustive and other secretin receptor ligands may be used.

The suitability of candidate ligands may be determined experimentally. For example, Charlton et al (1983) report that secretin injected intracerebroventricularly significantly increased defecation and decreased novel-object approaches in rats, but showed no significant effects on stereotypic behaviour. Such a test may be performed in rats with a secretin receptor ligand to determine its suitability for the present invention (i.e. those ligands which show similar effects via agonism of the secretin receptor may be selected).

Secretin is available from commercial sources (e.g. Peninsula Laboratories Inc, USA) or it and the above-described ligands may be obtained by reference to readily available published literature.

Compositions of the Invention.

The novel findings reported herein give rise to novel compositions which comprise a secretin receptor ligand together with at least one other compound active against CF. Such compounds include mucolytic agents such as acetylcysteine, deoxyribonuclease I (dornase) or erdosteine, as well as other anti-CF agents such as nedocromil or ibuprofen. The amount of secretin receptor ligand in such a composition may be, for example, from 1% to 99% by weight of the total amount of active ingredients (i.e. excluding carriers or diluents), for example from 10% to 90% by weight.

In a related aspect, the present invention provides a combination of a secretin receptor ligand and a second compound active against CF for simultaneous or sequential use in the treatment of CF. By "simultaneous" it is meant that the two compounds are administered at the same time, though not necessarily in the same composition. By "sequential" it is meant that the two compounds are administered within a time period such that the first of the two compounds is still active in the patient when administration of the second of the two compounds occurs. Preferably, "sequential" means within the same 24 hour, preferably within the same 12 hour, such as within the same 6, 3, 1, ½ or 1/4 hour time period.

Formulation and Administration.

Treatment of patients in accordance with the present invention may be performed by administering to a patient a secretin receptor ligand in the form of a pharmaceutical composition, either with or without a further active ingredient present reference below to compositions will be understood to include both types, though for brevity only the secretin receptor ligand is specifically mentioned . The composition may be in combination with a non-toxic, pharmaceutically acceptable carrier. In this context the invention also covers a method of treating CF comprising administering a therapeutically effective amount of the secretin receptor ligand of this invention or a composition of this invention on a patient to be treated.

In clinical practice the compositions of the present invention may be administered parenterally due to the fact that being a peptide the hormone is sensitive to biologically active environments. Oral or rectal administration may, however, be conceivable, for example using compositions of the slow release type making it possible for the active ingredient to reach the site of primary interest, namely the tertiary bronchus.

Secretin receptor ligands may be formulated in a suitable form for administration by inhalation e.g. via an aerosol or insufflation either through the mouth or nose , or by parenteral administration introduced by routes other than intestinal routes .

Delivery of proteins or peptides via inhalation may be

accomplished using liquid or solid preparations of the secretin receptor ligand. Thus the invention contemplates formulations comprising secretin receptor ligand for us in a wide variety of devices that are designed for the delivery of pharmaceutical compositions and therapeutic formulations to the respiratory tract. In one aspect of the present invention, secretin receptor ligand is administered in aerosolized or inhaled form. The secretin receptor ligand, combined with a dispersing agent, or dispersant, can be administered in an aerosol formulation as a dry powder or in a solution or suspension with a diluent.

Suitable dispersing agents are well known in the art, and include but are not limited to surfactants and the like. Surfactants are generally used in the art to reduce surface induced aggregation of protein caused by atomization of the solution forming the liquid aerosol. Examples of such surfactants include polyoxyethylene fatty acid esters and alcohols, and polyoxyethylene sorbitan fatty acid esters. Amounts of surfactants used will vary, being generally within the range of about 0.001 to 4% by weight of the formulation. In a specific aspect, the surfactant is polyoxyethylene sorbitan monooleate or sorbitan trioleate.

The liquid aerosol formulations contain the secretin receptor ligand and a dispersing agent in a physiologically acceptable diluent. The dry powder aerosol formulations of the present invention consist of a finely divided solid form of the secretin receptor ligand and a dispersing agent, and optionally a bulking

agent, such as lactose, sorbitol, sucrose, or mannitol, and the like, to facilitate dispersal of the powder. With either the liquid or dry powder aerosol formulation, the formulation must be aerosolized. That is, it must be broken down into liquid or solid particles in order to ensure that the aerosolized dose actually reaches the bronchii and/or alveoli, as desired. In general the mass median dynamic diameter will be 5 micrometers (μm) or less in order to ensure that the drug particles reach the lung bronchii or alveoli (Wearley, Crit. Rev. in Ther. Drug Carrier System **8**:333 (1991)).

With regard to construction of the delivery device, any form of aerosolization known in the art, including but not limited to nebulization, atomization or pump aerosolization of a liquid formulation, and aerosolization of a dry powder formulation, can be used in the practice of the invention. A delivery device that is uniquely designed for administration of solid formulations is envisioned. Often, the aerosolization of a liquid or a dry powder formulation will require a propellent. The propellent can be any propellent generally used in the art. Examples of useful propellents include chlorofluorocarbons, hydrofluorocarbons, hydrochlorofluorocarbons, and hydrocarbons, including trifluoromethane, dichlorodifluoromethane, dichlorotetrafluoroethanol, and 1,1,1,2-tetrafluoroethane, and combinations thereof.

In a preferred aspect of the invention, the device for aerosolization is a metered dose inhaler. A metered dose

inhaler provides a specific dosage when administered, rather than a variable dose depending on administration. Such a metered dose inhaler can be used with either a liquid or a dry powder aerosol formulation.

Systems of aerosol delivery, such as the pressurized metered dose inhaler and the dry powder inhaler are disclosed in Newman, Aerosols and the Lung, Clarke, S.W. and Davia, D. editors, pp 197-22 and can be used in connection with the present invention.

Additional pharmaceutical methods may be employed to control the duration of action of the antagonists of this invention. The antagonists also may be entrapped in microcapsules prepared, for example, by coacervation techniques by interfacial polymerization (for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate)microcapsules, respectively), in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules), or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences, 16th edition, Osol, A., ed (1980).

For intranasal administration, the secretin receptor ligands may be formulated as solutions for administration via a suitable metered or unit device or alternatively as a powder mix with a suitable carrier for the administration using a suitable delivery device. Alternatively, secretin receptor ligands could be delivered transnasally in a similar fashion. For example,

preparation of secretin for transnasal administration has been described in JP60123426.

Preparations for parenteral administration includes sterile aqueous or non-aqueous solutions, suspensions or emulsions. Examples of non-aqueous solvents or suspending media are propylene glycol, vegetable oils, such as olive oil, and injectible organic esters, such as ethyl oleate. These compositions may also contain adjuvants, such as preserving, wetting, emulsifying and dispersing agents. They may be sterilized, for example, by filtration through a bacteria-retaining filter, by incorporation of sterilizing agents in the composition, by irradiation or by heating. They may be also be manufactured in the form of sterile solid compositions, which can be dissolved in a sterile injectible medium immediately before use. As well as the more customary intravenous and intramuscular routes the compositions may also be administered by intraarticular injection.

The percentages of active ingredient in the compositions of the invention may be varied as long as they constitute a proportion such that a suitable dosage for the desired stimulatory effect on the pancreas is obtained. Obviously several unit dosage forms may be administered at about the same time. Generally, the compositions should contain from about 0.1% to about 80% by weight of active ingredient.

The dose employed depends upon the desired stimulatory effect

the route of administration and the duration of the treatment. Typical doses may be in the range of from $10^{-\text{[illegible]}}$ to $10^{-\text{[illegible]}}$ mg per day, preferably from $10^{-\text{[illegible]}}$ to 10^{-4} mg per day for a human patient. The secretin receptor ligand may be administered each day or, according to the wishes of the medical practitioner, less often, e.g. weekly, or until the desired therapeutic effect is achieved.

The following examples illustrate the invention.

RNA Expression Profiles.

Messenger RNA expression profiles of the secretin receptor (protein accession P47872; nucleotide accession U28281) was examined. Total RNA was isolated from tertiary / quaternary bronchus and lung parenchyma from 5 control and 5 CF donors using TriZol™, a commercially available solution of phenol and guanidine isothiocyanate, according to the protocol described by the manufacturer (Life Technologies). Samples of RNA were used only if intact 18s and 28s ribosomal RNA were detected by gel electrophoresis and if genomic DNA formed less than 10% of the total nucleic acid sample. Total RNA samples were annealed to the primer probe sequence plus a glyceraldehyde-3-phosphate dehydrogenase (GAPDH; accession no. P04406) primer and reverse transcribed using MuLV reverse transcriptase. Quantitative sequence detection was carried out on the resulting cDNA.

The applicants have developed protocols for quantitative

analysis of mRNA expression using the ABI prism 7700 Sequence Detection System (Perkin Elmer). Details of the system are set out in WO00/05409. In brief, the system uses fluorogenic probes to generate sequence specific fluorescent signals during PCR. The probes are oligonucleotides with fluorescent reporter and quencher dyes attached. While a probe is intact, the intensity of reporter fluorescence is suppressed by a quencher. When a probe forms part of a replication complex during the PCR process, the quencher is separated from the reporter dye resulting in a increase in fluorescence which is then detected by the ABI 7700 sequence detector. The ABI 7700 has a built in thermal cycler, and a laser directed at each of the 96 sample wells via bi-directional fibre optic cables. Emitted fluorescence through the cables to a detector where emissions which fall between 520nm and 660nm are collected every few seconds. The system software analyses the contribution of each component dye to the experiment spectrum, and normalises the signal to an internal reference dye. The peaks of these normalised 'reporter' values (Rn) are then plotted against thermal cycle number to produce an amplification plot - to allow visualisation of the extent of PCR product generation.

The starting copy number of a target sequence (Cn) is established by determining the fractional PCR cycle number (Ct) at which a PCR product is first detected - the point at which the fluorescence signal exceeds a threshold baseline. Therefore the lower a Ct value the greater the Cn. Quantification of the amount of target mRNA in each sample is established through

comparison of the experimental Ct values with standard curves for the target sequence which are constructed during each experiment.

Primer probe sets were specifically designed for the detection of secretin receptor mRNA. Off-line homology searches revealed no significant matches with gene sequences logged at Genbank. Forward and reverse primer and probe sequences for the secretin receptor were as follows :

Forward	GACCAGCATCATCTGAGAGGCT
Reverse	CCTTCGCAGGACCTCTCTTG
Probe	TCTCTGTCCGTGGGTGACCCTGCT

GAPDH primer probe sets were as follows

Forward	GAAGGTGAAGGTCGGAGTCAAC
Reverse	CAGAGTTAAAAGCAGCCCTGGT
Probe	TTTGGTCGTATTGGGCGCCT

Reaction conditions were optimised using genomic DNA as a template and a primer probe concentration grid followed by a probe concentration gradient experiment. Primer concentrations were selected to give the most efficient amplification of gene product. I.e. those which generate a low threshold cycle and a relatively high accumulation of fluorescence. These optimal primer concentrations were then used to select the optimum probe concentration.

A respiratory disease association of the secretin receptor was demonstrated by profiling secretin receptor mRNA expression in the tertiary bronchus and parenchyma from up to 5 fully consented donors pathologically and histologically diagnosed with the following respiratory disorders : non-smoker control, smoker, asthmatic, cystic fibrosis, pneumonia, emphysema, chronic obstructive pulmonary disease COPD . CF lung tissue was obtained by full consent from 5 patients undergoing heart and lung transplants.

Figure 2 shows the differential mRNA expression of the secretin receptor in control and CF lung regions. Data are representative of the mean±s.e.m QRT-PCR threshold cycle from 5 control and 5 cystic fibrosis tissue donors in each lung region. * $p=0.0246$ denotes statistical significance derived from an unpaired Students T-test. As a control, Figure 3 shows mRNA expression of GAPDH in control and CF lung regions. Data are representative of the mean±s.e.m QRT-PCR threshold cycle from 5 control and 5 cystic fibrosis tissue donors in each lung region. No statistical differences were observed within or between groups.

Decreased secretin receptor expression was demonstrated in the lung parenchyma of 5 COPD donors in comparison to 5 control donors $p=0.0465$. However no other donor groups exhibited differences in the expression of secretin receptor mRNA.

Biochemical and Physiological basis of action.

Impaired Cl^- efflux from cells in the respiratory tract into the airway lumen represents the etiological problem in CF. However, this loss of the Cl^- channel and ion movement also impairs bicarbonate (HCO_3^-) secretion from cells and enhances sodium ion (Na^+) reabsorption into cells, via epithelial, amiloride-sensitive Na^+ channels.

The lavage of the healthy lung consists primarily of H_2O (approx. 95%), with luminal HCO_3^- maintaining secreted proteins such as mucus and digestive enzymes in a soluble, inactive state. However, CF airway epithelia exhibit abnormally high rates of surface liquid absorption due to the high intracellular concentrations of Na^+ and Cl^- and therefore patients have a very low moisture content within their airways. Together this leads to significant thickening of the mucus, and subsequent impairment of the mucociliary clearance from the CF lung.

Movement of HCO_3^- across apical membrane of lung epithelial cells occurs predominantly via an electrogenic Cl^-/HCO_3^- exchanger, with water crossing hydrophobic plasma membranes either by simple osmotic diffusion or through a facilitative transport mechanism mediated by members of a family of aquaporin (AQP) water channel proteins. Currently it is thought that HCO_3^- and Cl^- are predominantly involved in the osmotic movement of H_2O.

Based on the physiological role of secretin and its receptor in ionic regulation in the duodenum and pancreas, the applicants suggest, based on the present findings, that increased mRNA and

functional expression of the secretin receptor may represent the human body's evolutionary, pathophysical response in order to compensate for the defect in the CFTR. As secretin peptide synthesis occurs in the duodenum, secretin receptors within the lung will not be exposed to the secretin peptide. While not being bound by any one particular theory, it is proposed that agonism of the secretin receptor by pharmacological intervention will treat the underlying biochemical respiratory problems associated with CF by all or some of the following:

- Stimulating Cl^- efflux via cAMP-dependent activation of Cl^- channels from respiratory cells of the tertiary bronchus. Secretin receptor stimulation or forskolin-mediated increases in cAMP have been shown to stimulate a small, single channel Cl^- selective conductance, of about 4pS across the apical membrane of rat pancreatic duct cells (Gray *et al*, 1988). Although secretin has been demonstrated to stimulate the CFTR and Cl^- efflux across the apical membranes of non-CF human epithelial cells (e.g. gallbladder; Dray-Charier *et al*, 1995), this Cl^- conductance is reported to be 6-12pS. Therefore this Cl^- represents an alternative cAMP-dependent Cl^- conductance.

- Stimulated increases in cAMP, activating protein kinases, and leading to the phosphorylation and subsequent regulation of epithelial Na^+ channels or Na^+-K^+-ATPases in respiratory cells, thereby reducing Na^+ reabsorption and stimulation of lung liquid movement. Such a mechanism has been demonstrated in the rat alveolar epithelial cells with cAMP coupled beta-adrenergic

receptor stimulation (Minakata *et al*, 1998).

- Subsequently increased luminal levels of Cl^- will act as a substrate for the secretin activated Cl^-/HCO_3^- exchanger, allowing the electrogenic movement of HCO_3^- into the airway lumen. Secretin has been widely demonstrated to stimulate the activity of Cl^-/HCO_3^- exchanger which is functionally coupled with a cAMP-dependent Cl- channel (CFTR) on the apical epithelium (for example in bile duct epithelial cells, Alvaro *et al*, 1993; 1997). This ionic movement mediated by secretin has been demonstrated to stimulate electrogenic Na^+/HCO_3^- cotransport, leading to correction of intracellular pH (Ishiguro *et al*, 1993).

- Additionally, increased HCO_3^- levels are known to maintain secreted proteins in mucus in a soluble, inactive state (Lee *et al*, 1999).

- Induce the translocation and insertion of AQPs into the plasma membrane, allowing the movement of water into the lumen of the airways. In rat cholangiocytes, secretin has been demonstrated to cause a 60 % concentration dependent increase in osmotic H_2O permeability by inducing the translocation of AQP-1 water channels (Marinelli *et al*, 1997). This process will also be assisted by the osmotic diffusion of H_2O across the plasma membrane, due to the correction of Na^+, Cl^-, HCO_3^- and pH via the previously described mechanisms, in bronchial cells and the

airway lumen

In summary, stimulation of the secretin receptor may be used to correct the ionic and H_2O problems of CF, reducing the thickness of the mucus layer, and allowing mucociliary clearance from the lung.

References

Alvaro, D., Cho, W.K., Mennone, A. & Boyer, J.L. 1993 Effects of secretin on intracellular pH regulation in isolated rat bile duct epithelial cells. *J. Clin. Invest.* **92**; 1314-1325

Alvaro, D., Gigliozzi, A., Fraioli, F., Romeo, R., Papa, E., Delle, Monache, M & Capocaccia, L. 1997 Hormonal regulation of bicarbonate secretion in the biliary epithelium. *Yale J. Biol.* **70**; 417-426

Charlton, C. G., et al 1983 Secretin modulation of behavioural and physiological functions in the rat. *Peptides*, **4**; 73942

Dray-Charier, N., Paul, A., Veissiere, D., Mergy, M., Scoazec, J.Y., Capeau, J., Brahimi-Horn, C. & Housset, C. 1995 Expression of cystic fibrosis transmembrane conductance regulator in human gallbladder epithelial cells. *Lab Invest* **73**; 828-836

Gardner, J.D., Rottman, A.J., Natarajan, S. & Bodansky, M. (1979) Interaction of secretin 5-27 and its analogues with hormone receptors on pancreatic acini. *Biochim Biophys Acta.* **583**; 491-503

Gray, M.A., Greenwell, J.R. & Argent, B.E. (1988) Secretin-regulated chloride channel on the apical membrane of pancreatic duct cells. *J Memb Biol.* **105**; 131-142

Haffer, B.M., Hocart, S.J., Coy, D.H., Mantey, S., Chiang, H.C. & Jensen, R.T. (1991) Reduced peptide bond pseudopeptide analogues of secretin. A new class of secretin receptor antagonists. *J. Biol. Chem.* **266**; 316-322

Ishiguro, H., Steward, M.C., Lindsay, A.R. & Case, R.M. (1996) Accumulation of intracellular HCO_3^- by Na^+/HCO_3^- cotransport in interlobular ducts from guinea-pig pancreas. *J. Physiol.* **495**; 169-178

Konig, W., Bickel, M., Karch, K., Teetz, V. & Uhmann. (1984) Analogues and fragments of secretin. *Peptides* **5**; 189 193

Lee, M.G., Wigley, W.C., Zeng. W., Noel, L.E., Marino, C.R., Thomas, P.J. & Muallem, S. (1999) Regulation of Cl^-/HCO_3^- exchange by cystic fibrosis transmembrane conductance regulator expressed in NIH3T3 and HEK293 cells. *J. Biol. Chem.* **274**; 3414-3421

Marinelli, R.A., Pham, L., Agre, P. & LaRusso, N.F 1997 Secretin promotes osmotic water transport in rat cholangiocytes by increased aquaporin-1 water channels in plasma membrane. *J. Biol. Chem.* **272**; 12984-12988

Minakata, Y., Suzuki, S., Grygorczyk, C., Dagenais, A & Berthiaume, Y. 1998 Impact of beta-adrenergic agonist on Na^+ channel and Na^+K^+-ATPase expression in avleolar type II cells. *Am. J. Physiol.* **275**; 414-422

Staun-Olsen, P., Ottesen, B., Gammeltoft, S. & Fahrenkrug, J. 1986 VIP binding sites on synaptosomes from rat cerebral cortex: structure-binding relationship. *Peptides* 7 *Suppl 1*; 181-186

Waelbroeck, M., Robberecht, P., De Neef, P., Chatelain, P. & Christophe, J. 1981 Binding of vasoactive intestinal peptide and its stimulation of adenylate cyclase through two classes of receptors in rat liver membranes. Effect of 12 secretin analogues and 12 secretin fragments. *Biochim Biophys Acta* **678**; 83-90

CLAIMS

1. A method of treatment of cystic fibrosis in a patient suffering from CF, the method comprising administering to said patient an effective amount of a secretin receptor ligand.

2. Use of a secretin receptor ligand for the manufacture of a medicament for the treatment of cystic fibrosis.

3. A method according to claim 1 or use according to claim 2 wherein said secretin receptor ligand is human secretin as shown in Figure 1.

4. A method according to claim 1 or use according to claim 2 wherein said secretin receptor ligand is administered by inhalation.

Figure 1

Mammalian Secretins

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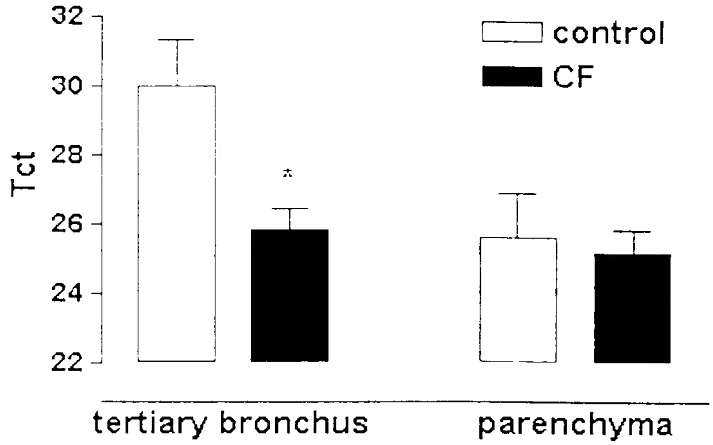
                 1         5                   10                  15
Human:      His-Ser-Asp-Gly-Thr-Phe-Thr-Ser-Glu-Leu-Ser-Arg-Leu-Arg-Glu-
Porcine:    His-Ser-Asp-Gly-Thr-Phe-Thr-Ser-Glu-Leu-Ser-Arg-Leu-Arg-Asp-
Canine:     His-Ser-Asp-Gly-Thr-Phe-Thr-Ser-Glu-Leu-Ser-Arg-Leu-Arg-Glu-

                             20                  25
Human:      Gly-Ala-Arg-Leu-Gln-Arg-Leu-Leu-Gln-Gly-Leu-Val-NH2
Porcine:    Ser-Ala-Arg-Leu-Gln-Arg-Leu-Leu-Gln-Gly-Leu-Val-NH2
Canine:     Ser-Ala-Arg-Leu-Gln-Arg-Leu-Leu-Gln-Gly-Leu-Val-NH2
```

Figure 2



32
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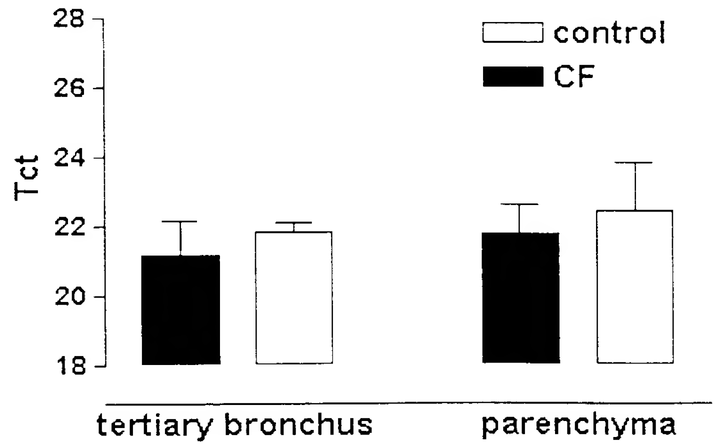
Tct
*
control
CF
tertiary bronchus
parenchyma

Figure 3



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Tct
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parenchyma